AMENDMENT TO THE SPECIFICATION

Sequence Listing

Please insert the Sequence Listing consisting of 11 sheets, provided herewith, into the application. The Sequence Listing is provided, as required, to comply with the requirements of 37 C.F.R. § 1.821 through 1.825. I hereby submit that the Sequence Listing contains no new matter.

On page 1, line 10, following the paragraph entitled CROSS REFERENCE TO RELATED APPLICATIONS, please insert the following paragraph:

REFERENCE TO SEQUENCE LISTING ON COMPACT DISC

This application refers to a "Table of Figure 16 listings", which is provided as an electronic document on two identical compact discs (CD-R), labeled "Copy 1" and "Copy 2." These compact discs each contain the file "50201.003002 Listing of Figure 163.txt" (3,676,752 bytes, created on August 3, 2004), which is incorporated by reference in its entirety.

Please also amend the specification on page 32, line 25, through page 33, line 19, as follows:

Information from the three-dimensional structure of FXIcat can be used in a variety of methods for identifying, manufacturing, or evaluating Factor XIa ligands. In one such aspect, the invention includes a method of selecting or designing a candidate ligand for Factor XIa. This method involves generating in a computer a three-dimensional structure of FXIcat based on the atomic coordinates in one or more of the files listed in Figure 16, or surrogates thereof, of the non-hydrogen atoms of residues serine 195, histidine 57, and aspartic acid 102 or atomic coordinates that have a root mean square deviation from the backbone atoms of the residues of less than 0.6, 0.5, 0.4, 0.3, 0.2, or 0.1 Å. A candidate ligand that has sufficient surface complementary to the structure to bind Factor XIa (e.g., binding in an aqueous solution such as an aqueous solution at pH 7.0) is designed, selected or identified. Surface complementarities can be assessed with a number of commercially available software packages, including, but not limited to, INSIGHT II® and QUANTA® ~~Insight II and Quanta~~ (Accelrys Inc: P.O. Box 5350, Princeton, NJ 08543-5350, USA), DOCK (Gschwend, D.A. and I.D. Kuntz, *J. Comput-Aided Mol. Design*, 1996) and SYBYL® ~~Sybyl~~ (Tripos, Inc.: 1699 South Hanley Rd., St. Louis, MO, 63144-2913, USA; Han et al., Bioorg. Med. Chem. Lett, 10:39-43, 2000). Designed ligands are then manufactured. If desired, the ability of any candidate ligand designed by the methods described herein to bind Factor XIa *in vitro* or *in vivo* can be optionally measured to confirm this binding and/or to compare the affinity of the ligand to other Factor XIa ligands. Compounds so prepared can also be used to determine their ability to inhibit Factor XIa, using inhibitition assays

as described herein.

Please amend the specification on page 79, lines 10-13, as follows:

Ecotin was further purified using a POROS® HQ ~~POROS HQ~~ column (Applied Biosystems) on a BIOCAD® ~~BioCAD~~ 700E in buffer containing 5 mM $MgCl_2$ in 10 mM Tris-HCl, pH 7.8. Ecotin did not bind to the matrix under these conditions, while impurities were retained on the column.

Please amend the specification on page 79, line 16, through page 80, line 18, as follows:

Recombinant FXIcat for use in crystallography was purified from *Pichia pastoris* conditioned medium by one of two methods. In one method, the recombinant enzyme was collected from conditioned medium by ammonium sulfate precipitation. The medium was first brought to 50% of saturation by the addition of crystalline $(NH_4)_2SO_4$. The resulting slurry was mixed at room temperature for 30 minutes, and the precipitated proteins removed by centrifugation at 20,000 x *g* from 20 minutes. The supernatant solution was isolated and brought to 80% of saturation by the addition of $(NH_4)_2SO_4$. The slurry was again mixed at room temperature for 30 minutes, and the precipitated protein was collected by centrifugation as described above. The supernatant solution was discarded, and the precipitate was redissolved in a minimal amount of 20 mM Tris-HCl, pH 7.4. The redissolved protein was dialyzed against

two changes (at least 50 volumes each) of the same buffer at 4° C. The dialyzed protein was applied to a 2.6 x 8 cm Chelating SEPHAROSE™ ~~Sepharose~~ Fast Flow column (Amersham Bioscience, P/N 17-0575-01) charged with Zn^{+2}. The column was run at room temperature at a flow rate of ~8 mL/minutes. Following loading, the column was washed with additional 20 mM Tris-HCl, pH 7.4 until the A_{280} of the effluent returned to baseline. The column was eluted with a 250 mL gradient from 0 to 50 mM imidazole in 20 mM Tris-HCl, pH 7.4. The pH of the fraction containing eluted, recombinant FXIcat was adjusted to pH~6 by the addition of 1/200 volume of 20% CH_3COOH. The protein was then enzymatically deglycosylated by the addition of Endoglycosidase H (New England Biolabs, Cat. No. P0703S) to a final concentration of 500-1000 mU/mL. Digestion was carried out at room temperature for 1.5-2 hours. The pH of the solution was brought up to pH~7.4 by the addition of 1/200 volume of 2 M Tris, and the fraction was applied to a 1.6 x 8 cm SP-SEPHAROSE™ ~~SP-Sepharose~~ Fast Flow column (Amersham Bioscience, P/N 17-0729-01) equilibrated in 20 mM Tris-HCl, pH 7.4 at a flow rate of 8 mL/minutes. After the column was loaded, it was washed with additional 20 mM Tris-HCl, pH 7.4 buffer. The column was then washed with 0.1 M NaCl in the same buffer to remove minor contaminants. Recombinant FXIcat was eluted from the column with a step to 0.2 M NaCl in 20 mM Tris-HCl, pH 7.4.

Please amend the specification on page 80, line 19, through page 81, line 17, as follows:

Alternatively, various mutant forms of FXIcat were purified from concentrated, diafiltered *P. pastoris* conditioned medium. Cells and cell debris were removed by

centrifugation followed by filtration through 0.8 μm mixed ester cellulose nitrate filters. The filtered medium (7-15 L) was concentrated to ~1 L using a CENTRAMATE™ ~~Centramate~~ PE apparatus equipped with a 10K NMWCO membrane cassette (Pall-Gelman, P/N OS010C10). The concentrate was diluted with 4 L of 20 mM Tris-HCl, pH 7.4 and reconcentrated to ~1 L. The dilution and concentration were repeated once more to yield the final concentrated, diafiltered medium. Recombinant FXIcat was purified by a combination of cation exchange and heparin-affinity chromatography. The concentrated, diafiltered medium was applied directly to a 1.6 x 8 cm SP-SEPHAROSE™ ~~Sepharose~~ Fast Flow column equilibrated in 20 mM Tris-HCl, pH 7.4 at a flow rate of 8-10 mL/minutes. After the column was loaded, it was washed with additional 20 mM Tris-HCl, pH 7.4 buffer. The column was then washed with 0.1 M NaCl in the same buffer to remove minor contaminants. Recombinant FXIcat was eluted from the column with a step to 0.2 M NaCl in 20 mM Tris-HCl, pH 7.4. Recombinant FXIcat eluted from the SP-column was diluted 1:1 with 20 mM Tris-HCl, pH 7.4 and applied to a 5 mL HITRAP™ ~~HiTrap~~™ Heparin column (Amersham Bioscience, P/N 17-0407-01) equilibrated in 0.1 M NaCl; 20 mM Tris-HCl, pH 7.4 at a flow rate of 3-4 mLl/minutes. The protein was eluted from this column with a 50 mL gradient from 0.1 to 1 M NaCl in the same buffer. The purity and concentration of the enzyme was determined by RP-HPLC and SDS-PAGE. Typically, the concentration was ~1 mg/mL, and the purity was at least 95%. In some instances, a third purifcation step was included. The heparin column eluate was applied to a 2.6 x 90 cm SUPERDEX™ ~~Superdex~~ 75 column equilibrated in 20 mM Tris-HCl, pH 7.4; 0.2 M NaCl at a flow rate of 4-5 mL/minutes. The enzyme eluted between 70 and 90 minutes, consistent with an apparent molecular weight of 30,000.

Please amend the specification on page 86, lines 23-26, as follows:

FXIcat-ecotin complex was formed by incubating a stoichiometric quantity of each component at 4 °C overnight and subsequently isolated by chromatography on a SUPEROSE™ ~~Superose~~ 12 column (Amersham Bioscience) in 20 mM Tris-HCl, pH7.8 and 0.1 M NaCl on BIOCAD™ ~~BioCAD~~ 700E.

Please amend the specification on page 87, lines 11-22, as follows:

The structure of FXIcat-ecotin M84R was solved by molecular replacement using AMoRe ~~Amore~~. The search model was created from the coordinates of the tetrameric rat granzyme B [N66Q] - ecotin [81-84 IEPD] with PDB code 1FI8 (Waugh *et al.*, *Nat. Struct. Biol.* 7:762, 2000). The conserved core structure of serine proteases was identified first by structure alignment of several serine proteases. All the side chain atoms beyond C_β, all surface loops in granzyme B, and all water molecules were deleted from the model. A clear solution was obtained using data of 20-5 Å with correlation coefficient of 52.4 and R factor of 48.9%. This solution also persisted in calculations performed using data in different resolution ranges. Structure refinement was carried out using CNX™ ~~CNX~~ (Accelrys Inc., San Diego, California) and model building was performed in QUANTA® ~~Quanta~~ (Accelrys Inc., San Diego, California).

Please amend the specification on page 90, line 19, through page 96, line 14, as follows:

Example 11. Crystallization of FXIcat-Wild Type Ecotin Complex

The FXIcat-wild type ecotin complex was crystallized at room temperature by the hanging drop vapor diffusion method (McPherson, *Methods Biochem. Anal.* 23:249-345, 1976) from 0.1 M $(NH_4)_2SO_4$, 22% (w/v) PEGMME-2000 and 0.1 M Na cacodylate, pH 6.2. The crystals belong to the space group $P2_12_12_1$ with unit cell parameters of a=44.3 Å, b=91.9 Å, and c=186.2 Å. There are two FXIcat and two ecotin molecules forming a tetramer in the asymmetric unit, and the diffraction data extends to 2.6 Å. The coordinates of FXIcat-ecotin M84R were used for the calculation of the difference electron density map by the program CNX™ ~~CNX~~ (Accelrys Inc., San Diego, California) for FXIcat-wild type ecotin. Subsequent model building was carried out in the program QUANTA®2000 ~~Quanta2000~~ (Accelrys Inc., San Diego, California) and refinement was done by CNX™ ~~CNX~~.

Example 12. Crystallization of FXIcat-Ecotin IX-D Complex

The FXIcat-ecotin IX-D complexe was crystallized at 10°C by the hanging drop vapor diffusion method (McPherson, *Methods Biochem. Anal.* 23:249-345, 1976) from 0.1 M $(NH_4)_2SO_4$, 22% (w/v) PEGMME-2000 and 0.1 M Na cacodylate, pH 6.2. The crystals belong to the space group $P2_12_12_1$ with unit cell parameters of a=45.4 Å, b=91.4 Å, and c=188.6 Å. There are two FXIcat and two ecotin molecules forming a tetramer in the asymmetric unit, and the diffraction data extends to 3.0 Å. The coordinates of FXIcat-ecotin M84R were used for the

calculation of the difference electron density map by the program CNX™ ~~CNX~~ (Accelrys Inc., San Diego, California) for FXIcat-ecotin IX-D. Subsequent model building was carried out in the program QUANTA®2000 ~~Quanta2000~~ (Accelrys Inc., San Diego, California) and refinement was done by CNX™ ~~CNX~~.

Example 13. Crystallization of FXIcat-S**434**A,T**475**A,K**437**A with Benzamidine

The FXIcat triple mutant S**434**A, T**475**A, K**437**A was cloned and purified as described above. Benzamidine was added to the protein to a concentration of 1 mM and the complex was concentrated to about 18-20 mg/mL. Cubic crystals were obtained in 2.0 M ammonium sulfate and 0.1 M Tris-HCl, pH 7.5-9 at 20 °C. The crystals belong to the cubic space group I23 with unit cell parameters of a=b=c=120.1 Å. There is one FXIcat molecule in the asymmetric unit, and the X-ray diffraction data extends to 1.96 Å.

The initial solution of this structure was found by molecular replacement using an automated package for molecular replacement (AmoRe,: Navaza J, *Acta Cryst.* A50: 157-163, 1994). The search model was one FXIcat molecule from the tetramer structure of FXIcat-ecotin M84R complex. To avoid model bias, all the surface loops, the side chain atoms beyond C_β, and water molecules were removed from the model. A clear solution was obtained using data of 20-5 Å with correlation coefficient of 50.6 and R factor of 34.8%. Structure refinement was performed using CNX™ ~~CNX~~ (Accelrys Inc., San Diego, California), and model building was performed using QUANTA® ~~Quanta~~ (Accelrys Inc., San Diego, California)).

Example 14. Crystallization of FXIcat with SPRL-122599

A ten-fold molar excess of 10 mM SPRL-122599 in DMSO was added to the FXIcat quadruple mutant S**434**A, T**475**A, C**482**S, K**437**A, incubated at 4°C and then concentrated to 15 mg/mL. Cubic crystals were obtained using the hanging drop method (McPherson, *Methods Biochem. Anal.* 23:249-345, 1976) with 23% (w/v) PEG 4000, 0.2 M Li_2SO_4, 0.1 M Tris-HCl, pH 8.5 at 10 °C. The crystals belong to the space group I23 with unit cell parameters of a=b=c=121.5 Å. Diffraction data were measured to 2.8 Å resolution and processed by HKL2000. Phases were calculated using the structure described in Example 13 as the model, and CNX™ ~~CNX~~ (Accelrys Inc., San Diego, California) was used for calculating the difference electron density map.

Example 15. Crystallization of FXIcat with SPRL-121995

A ten-fold molar excess of 10 mM SPRL-121995 in DMSO was added to diluted FXIcat-S**434**A,T**475**A,C**482**S,K**437**A, incubated at 4 °C, and then concentrated to 15 mg/mL. Cubic crystals were obtained using the hanging drop method (McPherson, *Methods Biochem. Anal.* 23:249-345, 1976) with 24% w/v polyethylene glycol 4000, 0.16 M lithium sulfate, and 0.08 M Tris-HCl, pH 8.5 at 10 °C. The crystals belong to space group I23 with unit cell parameters of a=b=c=121.7 Å. Diffraction data were measured to 2.1 Å resolution and processed by HKL2000. Phases were calculated using the structure described in Example 13 as the model, and CNX™ ~~CNX~~ (Accelrys Inc., San Diego, California) was used for calculating the difference electron density map.

Example 16. Crystallization of FXIcat with SPRL-124336

A ten-fold molar excess of 10 mM SPRL-121995 in DMSO was added to diluted FXIcat-S434A,T475A,C482S,K437A, incubated at 4 °C, and then concentrated to 20 mg/mL. Cubic crystals were obtained using the hanging drop method (McPherson, *Methods Biochem. Anal.* 23:249-345, 1976) with 30% PEGMME 5000, 0.1 M Tris pH 7.9, 0.2 M $(NH_4)_2SO_4$, pH 8.5 at 10 °C. The crystals belong to space group P32 with unit cell parameters of a=b=41.9, c=103.8 Å. Diffraction data were measured to 3.0 Å resolution and processed by HKL2000. Phases were calculated using the structure described in Example 13 as the model, and CNX™ ~~CNX~~ (Accelrys Inc., San Diego, California) was used for calculating the difference electron density map.

Example 17. Crystallization of FXIcat with SPRL-123682

A ten-fold molar excess of 10 mM SPRL-123682 in DMSO was added to diluted FXIcat-S434A,T475A,C482S,K437A, incubated at 4 °C, and then concentrated to 15 mg/mL. Cubic crystals were obtained using the hanging drop method (McPherson, *Methods Biochem. Anal.* 23:249-345, 1976) with 18% (w/v) PEG 4000, 0.2 M Li_2SO_4, 0.1 M Tris-HCl, pH 8.5 at 10 °C. The crystals belong to space group I23 with unit cell parameters of a=b=c=121.3 Å. The diffraction data were measured to 2.0 Å resolution and processed by HKL2000. Phases were calculated using the structure described in Example 13 as the model, and CNX™ ~~CNX~~ (Accelrys Inc., San Diego, California) was used for calculating the difference electron density map.

Example 18. Crystallization of FXIcat with SPRL-123672

A ten-fold molar excess of 10 mM SPRL-123672 in DMSO was added to diluted FXIcat-S**434**A,T**475**A,C**482**S,K**437**A, incubated at 4 °C, and then concentrated to 15 mg/mL. Cubic crystals were obtained using the hanging drop method (McPherson, *Methods Biochem. Anal.* 23:249, 1976) with 2.0 M ammonium sulfate, 0.1 M Tris-HCl, pH 8.5 at 10 °C. The crystals belong to space group I23 with unit cell parameters of a=b=c=121.4 Å. Diffraction data were measured to 2.2 Å resolution and processed by HKL2000. Phases were calculated using the structure described in Example 13 as the model, and CNX™ ~~CNX~~ (Accelrys Inc., San Diego, California) was used for calculating the difference electron density map.

Example 19. Crystallization of FXIcat with SPRL-123545

A ten-fold molar excess of 10 mM SPRL-123545 in DMSO was added to diluted FXIcat-S**434**A,T**475**A,C**482**S,K**437**A, incubated at 4 °C, and then concentrated to 15 mg/mL. Cubic crystals were obtained using the hanging drop method (McPherson, *Methods Biochem. Anal.* 23:249, 1976) with 30% (w/v) PEG 4000, 0.2 M Li_2SO_4, 0.1 M Tris-HCl, pH 8.5. at 10 °C. The crystals belong to space group I23 with unit cell parameters of a=b=c=120.7 Å. Diffraction data were measured to 2.05 Å and processed by HKL2000. Phases were calculated using the structure described in Example 13 as the model, and CNX™ ~~CNX~~ (Accelrys Inc., San Diego, California) was used for calculating the difference electron density map.

Example 20. Crystallization of FXIcat with SPRL-122624

A ten-fold molar excess of 10 mM SPRL-122624 in DMSO was added to the FXIcat-S434A,T475A,C482S,K437A, incubated 30 minutes at 20 °C and then concentrated to 20 mg/mL. Cubic crystals were obtained using the hanging drop method (McPherson, *Methods Biochem. Anal.* 23:249-345, 1976) with 20% (w/v) PEG monomethyl ether 5000, 0.1 M ammonium sulfate, and 0.1 M Tris-HCl, pH 7.6 at 10 °C. The crystals belong to the space group I23 with unit cell parameters of a=b=c=122.4 Å. Diffraction data were measured to 2.05 Å and processed by HKL2000. Phases were calculated using the structure described in Example 13 as the model, and CNX™ ~~CNX~~ (Accelrys Inc., San Diego, California) was used for calculating the difference electron density map.

Example 21. Crystallization of FXIcat with SPRL-123586

A ten-fold molar excess of 10 mM SPRL-123586 in DMSO was added to the FXIcat-S434A,T475A,C482S,K437A, incubated 30 minutes at 4°C and then concentrated to 20 mg/mL. Needle-like crystals were obtained using the hanging drop method (McPherson, *Methods Biochem. Anal.* 23:249-345, 1976) with 1.4 M tri-sodium citrate, 0.1 M HEPES, pH 7.5 at 10 °C. The crystals belong to the space group P21 with unit cell parameters of a=55.8 Å, b=70.3 Å, c=62.1 Å, $\alpha = \gamma = 90°$ and $\beta = 102.2°$. Diffraction data were measured to 2.7 Å and processed by HKL2000. There are two FXIcat molecules in the asymmetric unit. The orientations of the two monomers were determined by AMoRe ~~AmoRe~~ using the structure described in Example 13 as

model. The solutions were refined in CNX™ ~~CNX~~ (Accelrys Inc., San Diego, California). The difference electron density map was also calculated in CNX.

Example 22. Soaking SPRL-130421 into preformed FXIcat-benzamidine crystal

The preformed FXIcat-benzamidine crystals were obtained in a condition similar to Example 13. Benzamidine was added to the FXIcat-S434A,T475A,C482S,K437A protein to a concentration of 1 mM and the complex was concentrated to 20 mg/mL. Cubic crystals were obtained by the hanging-drop method in 2.0 M ammonium sulfate and 0.1 M Tris-HCl, pH 8.5 at 10 °C. A preformed FXIcat-benzamidine crystal was transferred into a drop containing its mother liquor with 5 mM of SPRL-130421 and incubated at 10 °C overnight. The crystal is in the space group I23 with unit cell parameters of a=b=c= 121.0 Å. Diffraction data were measured to 1.9 Å and processed by HKL2000. Phases were calculated using the structure of FXIcat-SPRL-123545 described in Example 19 as the model, and CNX™ ~~CNX~~ (Accelrys Inc., San Diego, California) was used for calculating the difference electron density map. Model building was performed using QUANTA® ~~Quanta~~ (Accelrys Inc., San Diego, California).

Please amend the specification on page 150, lines 12-20, as follows:

A Schlenk tube was flame dried under high vacuum, backfilled with Argon, and charged with CuI (110 mg), NaCN (575 mg), and $Pd(PPh_3)_4$ (335mg). Compound XLII (2.5g) was dissolved in dry acetonitrile (15 mL) and added to the Schlenk flask via syringe. The solution

was stirred vigorously at 80°C for 1.5 hours. The reaction mixture was then cooled to room temperature, diluted with ethyl acetate, filtered through CELITE® ~~Celite~~, and the resulting filtrate was washed with water and brine. After drying with $MgSO_4$, the organic layer was concentrated to provide 1.42 g of 8-benzyloxycarbonylamino napththalene-2-carbonitrile which was subsequently utilized without further purification.

Please amend the legend to Table 11 on page 161, lines 3-5, as follows:

(b) Purity estimated by HPLC analysis, 5 minute gradient elution with 0%-10% acetonitrile to 90-100% acetonitrile/water, 0.1% TFA, 1 mL/minute, PHENOMENEX® LUNA® ~~Phenomenex Luna~~ C18 column (30 x 3 mm, 4 micron).~~.~~

Please amend the specification on page 181, lines 1-8, as follows:

The final concentration of the substrate in the assay was 100 μM, and the final concentration of the enzyme was 0.25 nM. Inhibitors were tested by serial dilution over an appropriate range to yield a dose response curve for determination of the inhibitors' IC_{50} value. The assay mixture was incubated for 30 minutes at 30° C, and the fluorescent yield measured using a PE-Wallac ~~Victor 2~~ VICTOR2™ Plate Reader. Dose response curves were fit to equation 1 below in which A is the maximum inhibition, B is the minimum inhibition, C is the IC_{50}, and D is the Hill coefficient.

Please amend the specification on page 183, line 10, through page 184, line 25, as follows:

An activated partial thomboplastin time (aPTT) assay was used to measure the ability of compounds to inhibit the contact coagulation pathway. This pathway involves Factor XII, kallikrein, and Factor XI, which activates Factor IX and Factor VIII, leading to activation of Factor X and Factor V, and then activation of Factor II to form a blood clot (Figure 1). For this assay, $CaCl_2$ (30 mM) was placed in a large central reagent position of a Thromboscreen 400C instrument, allowing it to equilibrate to $37^{O}C$. Plasma (50 ul) and compounds of the invention were added at different concentrations to cuvettes. After incubation for two minutes, aPTT reagent (ALEXIN® ~~ALEXIN~~, Sigma) was added (50 ul) and incubated an additional three minutes. The cuvettes were transferred to a measuring position; prewarmed $CaCl_2$ reagent (50 ul) was added, and readings were then taken over a maximum of 300 seconds. A dose response curve was generated, and the concentration at which the clotting time was doubled (2 x aPTT) was determined. Compounds which inhibit Factor XIa in the desired range desirably have an effect at less than 50 uM, more desirably at less than 10 uM.

A prothrombin time (PT) assay was also used to measure inhibition of coagulation. In this assay, the Factor XI dependent steps are bypassed. Hence, the assay measures inhibition of Factor VIIa, Factor Xa, and thrombin, but not FXI. This assay measures the ability of Factor VIIa to activate Factor X, which activates Factor II to form a blood clot. For this assay, the

thromboplastin reagent (THROMBOMAX® ~~ThromboMax~~ with Calcium, Sigma) was placed in a central reagent position in a Thromboscreen 400C instrument, and allowed to equilibrate to 37 ^{O}C. Plasma (50 ul of plasma prewarmed for three minutes) and compounds of the invention (different concentrations) were added to cuvettes. The cuvettes were transferred to a measuring position. The prewarmed Thromboplastin reagent (100 ul) was then added, and readings were then taken over 300 seconds. A dose response curve was generated, and the concentration at which the clotting time was doubled (2 x PT) was determined.

Please amend the specification on page 185, lines 6-22, as follows:

Briefly, male Sprague-Dawley rats (300 – 400 g) were anesthetized using ketamine/xylazine I.M. A midline laparotomy was performed, and the vena cava was visualized and gently separated from the descending aorta using cotton swabs. Both iliolumbar veins were ligated with suture (3-0 silk). A loose ligature (3-0 silk) was placed around the vena cava distal to the renal veins. A 25G butterfly bent at a 90-degree angle and attached to a 5 cc syringe filled with 2.5 mL of hypotonic saline (0.225%) was inserted into the vena cava at the level of the iliolumbar veins and glued into place using VETBOND™ ~~Vetbond™~~. A 26G needle bent to a 90-degree angle was then tied tightly to the vena cava, using the loose ligature. An atraumatic clamp was placed on the vena cava proximal to the iliac bifurcation, and the hypotonic saline manually infused at a rate of 10 mL/minutes. The atraumatic clamp was removed, along with the 26G needle, creating a 26G stenosis. Organs were carefully replaced, and animals were kept

normothermic until sacrifice. Animals were sacrificed by anesthetic overdose at 10 or 60 minutes post thrombus induction. Following sacrifice, the vena cava was isolated using hemostats, and the clot(s) was dissected out. Clots were removed, blotted on gauze, and then immediately weighed using an analytical balance.